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Authors

Khamaikawin, Wannisa
Shimizu, Saki
Kamata, Masakazu
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Modeling Anti-HIV-1 HSPC-Based Gene Therapy in Humanized Mice Previously Infected with HIV-1

Wannisa Khamaikawin,[2,4,7] Saki Shimizu,[1,2,4,7] Masakazu Kamata,[1,7] Ruth Cortado,[2,4] Yujin Jung,[2,4] Jennifer Lam,[2,4] Jing Wen,[3,4] Patrick Kim,[1,2,3,4] Yiming Xie,[3,4] Sanggu Kim,[3,4] Hubert Arokium,[3,4] Angela P. Presson,[5,6] Irvin S.Y. Chen,[1,3,4] and Dong Sung An[1,2,4]

[1]Division of Hematology-Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA; [2]School of Nursing, University of California, Los Angeles, Los Angeles, CA 90095, USA; [3]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA; [4]UCLA AIDS Institute, Los Angeles, CA 90095, USA; [5]Department of Biostatistics, University of California, Los Angeles, Los Angeles, CA 90095, USA; [6]Division of Epidemiology, University of Utah, Salt Lake City, UT 84132, USA

Investigations of anti-HIV-1 human hematopoietic stem/progenitor cell (HSPC)-based gene therapy have been performed by HIV-1 challenge after the engraftment of gene-modified HSPCs in humanized mouse models. However, the clinical application of gene therapy is to treat HIV-1-infected patients. Here, we developed a new method to investigate an anti-HIV-1 HSPC-based gene therapy in humanized mice previously infected with HIV-1. First, humanized mice were infected with HIV-1. When plasma viremia reached >10^7 copies/mL 3 weeks after HIV-1 infection, the mice were myeloablated with busulfan and transplanted with anti-HIV-1 gene-modified CD34$^+$ HSPCs transduced with a lentiviral vector expressing two short hairpin RNAs (shRNAs) against CCR5 and HIV-1 long terminal repeat (LTR), along with human thymus tissue under the kidney capsule. Anti-HIV-1 vector-modified human CD34$^+$ HSPCs successfully repopulated peripheral blood and lymphoid tissues in HIV-1 previously infected humanized mice. Anti-HIV-1 shRNA vector-modified CD4$^+$ T lymphocytes showed selective advantage in HIV-1 previously infected humanized mice. This new method will be useful for investigations of anti-HIV-1 gene therapy when testing in a more clinically relevant experimental setting.

INTRODUCTION

Hematopoietic stem/progenitor cell (HSPC)-based gene therapy to reconstitute an HIV-1-infected patient's immune system with genetically engineered, HIV-resistant HSPCs and their progenies has the potential to provide a long-term HIV-1 control without daily drug treatment or to achieve an HIV/AIDS cure.[1–7] Similar to the one case of an HIV-1 cure achieved by transplants of CCR5Δ32/Δ32 homozygous HIV-1-resistant donor bone marrow (BM) cells in an HIV-infected patient,[8–11] anti-HIV-1 HSPC gene therapy aims to treat an HIV-1-infected patient by repopulating HIV-1-resistant HSPCs and their progenies.

Various anti-HIV-1 genes have been developed to genetically protect human CD34$^+$ HSPCs and their progenies.[4,5,12–29] Previously, *in vivo* investigations of these anti-HIV-1 genes have been performed by viral challenge after transplantation of anti-HIV-1 gene-modified HSPCs in immunodeficiency mice.[15,30–38] The experimental design allowed us to examine the engraftment of anti-HIV-1 gene-modified HSPCs without confounding HIV-1 pathogenic effects. Subsequently, the mice are challenged with HIV-1 to examine the protection of anti-HIV-1 gene-modified immune cells. Using this protocol, we demonstrated that human CD34$^+$ HSPCs transduced by an anti-HIV-1 lentiviral vector expressing dual short hairpin RNAs (shRNAs) targeting CCR5 (sh1005) and HIV-1 long terminal repeat (LTR) (sh516) successfully reconstituted anti-HIV-1 vector-modified multi-lineage hematopoietic cells in humanized BM , liver, and thymus (BLT)-transplanted mice; we also demonstrated the selective advantage of the anti-HIV-1 dual shRNA gene expressing CD4$^+$ T lymphocytes after an HIV-1 challenge in the reconstituted mice.[15] However, it is unknown whether the anti-HIV-1 gene-modified HSPCs can engraft, differentiate into mature hematopoietic cells, and be protected in humanized mice previously infected with HIV-1.

In this report, our primary goal was to establish a new method to examine an anti-HIV-1 HSPC-based gene therapy strategy in humanized mice previously infected with HIV-1. First, we prepared humanized mice by transplanting human fetal liver-derived CD34$^+$ HSPCs into irradiated neonatal NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Humanized mice were subsequently infected with HIV-1. When plasma viremia reached >10^7 copies/mL, HIV-1-infected humanized mice were preconditioned with busulfan and transplanted

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[7]These authors contributed equally to this work.

Correspondence: Dong Sung An, School of Nursing, University of California, Los Angeles, 188 BSRB, 615 Charles E. Young Dr. South, Los Angeles, CA 90095, USA.
E-mail: an@ucla.edu

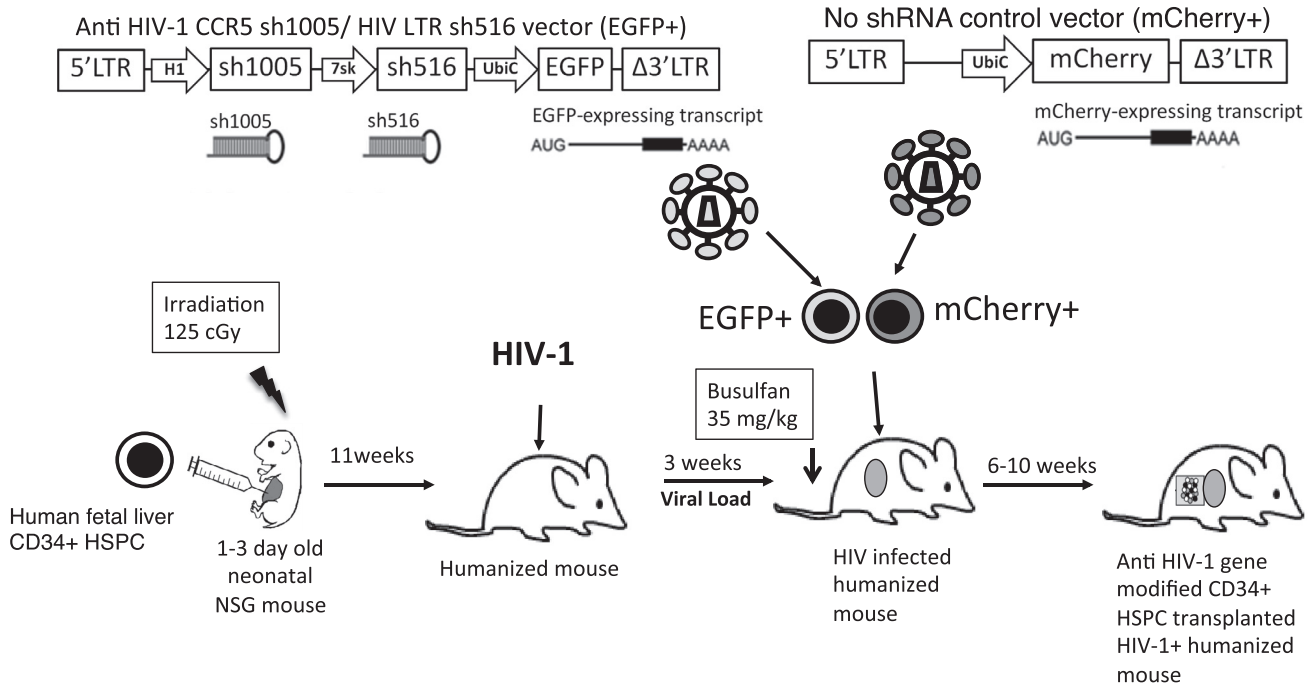

Figure 1. A New Method to Test an Anti-HIV-1 HSPC-Based Gene Therapy in Humanized Mice Previously Infected with HIV-1

Neonatal NSG mice (1–3 days old) were irradiated at 125 cGy and transplanted with human fetal liver-derived $CD34^+$ HSPCs (0.5×10^5–1.0×10^5 cells per mouse) by an intrahepatic injection. Human hematopoietic cell reconstitution was examined in peripheral blood at 10 weeks. Humanized mice were infected with CCR5-tropic HIV-1_{NFNSX} at a dose of 200 ng of p24 through retro-orbital vein plexus at 11 weeks. Plasma viral load was measured at week 3 post-HIV-1 infection. Humanized mice were pre-conditioned with busulfan (35 mg/kg) for myeloablation by an intraperitoneal injection. Human fetal liver-derived $CD34^+$ HSPCs (0.5×10^6) were transduced with an anti-HIV-1 lentiviral vector expressing short hairpin RNAs against human CCR5 (sh1005) and HIV-1 LTR (sh516) expressing EGFP. The other half of the $CD34^+$ HSPCs (0.5×10^6) were transduced with a control lentiviral vector expressing mCherry without shRNA. Transduced $CD34^+$ HSPCs were mixed after the vector transduction and transplanted by a retro-orbital vein plexus injection and with a piece of human thymus under the mouse kidney capsule. Reconstitution of vector-modified human hematopoietic cells was examined in peripheral blood 6–10 weeks and in lymphoid tissues 12 weeks after vector-modified $CD34^+$ HSPC transplant.

with anti-HIV-1 gene-modified, human fetal liver-derived $CD34^+$ HSPCs and human thymus tissue from the same donor to repopulate HIV-1-resistant, gene-modified immune cells in lymphoid organs in the HIV-1 previously infected humanized mice. Using this new experimental method, we evaluated the engraftment of the dual shRNAs, CCR5 (sh1005) and HIV-1 LTR (sh516), modified HSPCs, differentiation into mature hematopoietic cells, tissue reconstitution, and the selective advantage of $CD4^+$ T lymphocytes in the HIV-1 previously infected humanized mice.

RESULTS

Preparation of HIV-1-Infected Humanized NSG Mice

We investigated a new method to test anti-HIV-1 HSPC-based gene therapy in humanized mice previously infected with HIV-1 (Figure 1). To reconstitute HIV-1 target human hematopoietic cells, neonatal NSG mice (1–3 days old) were irradiated (125 cGy) and transplanted with human fetal liver-derived $CD34^+$ HSPCs from 5 donors (0.5×10^5–1.0×10^5 cells per mouse) by an intrahepatic injection. Human multi-lineage hematopoietic cells ($CD45^+$ hematopoietic, $CD3^+$ T lymphocyte and $CD19^+$ B lymphocyte, and $CD4^+$ T lymphocyte and $CD8^+$ T lymphocyte populations) were reconstituted in peripheral blood 10 weeks after $CD34^+$ HSPC transplant, determined by monoclonal antibody staining and multi-color flow cytometric analysis (Figure 2). Humanized mice were divided to create 2 groups with similar percentage levels of $CD45^+$, $CD3^+$ $CD19^+$, and $CD4^+$ $CD8^+$ cell reconstitution and equally divided among donors (Figure 2, lower panels). Group 1 mice (n = 11) were infected with CCR5-tropic HIV-1_{NFNSX} (200 ng of p24 Gag) intravenously via retro-orbital vein plexus 11 weeks after $CD34^+$ HSPC transplant. Group 2 mice (n = 12) were used as a non-HIV-1-infected control. Plasma viral load reached an average of 1.25×10^7 copies/mL ± 1.48×10^7 SD 3 weeks after HIV-1 injection.

Transplantation of Anti-HIV-1 Gene Vector-Transduced HSPCs in HIV-1 Previously Infected Humanized Mice

HIV-1-infected (n = 11) and HIV-1-uninfected (n = 12) humanized mice were myeloablated by an intraperitoneal injection of busulfan (35 mg/kg) 3 weeks after HIV-1 injection. To avoid potential problems caused by human leukocyte antigen (HLA) mismatch, cryopreserved, human fetal liver-derived $CD34^+$ HSPCs from the same donors were thawed and transduced with the anti-HIV-1 dual shRNA sh1005/sh516 vector (EGFP marked) or the

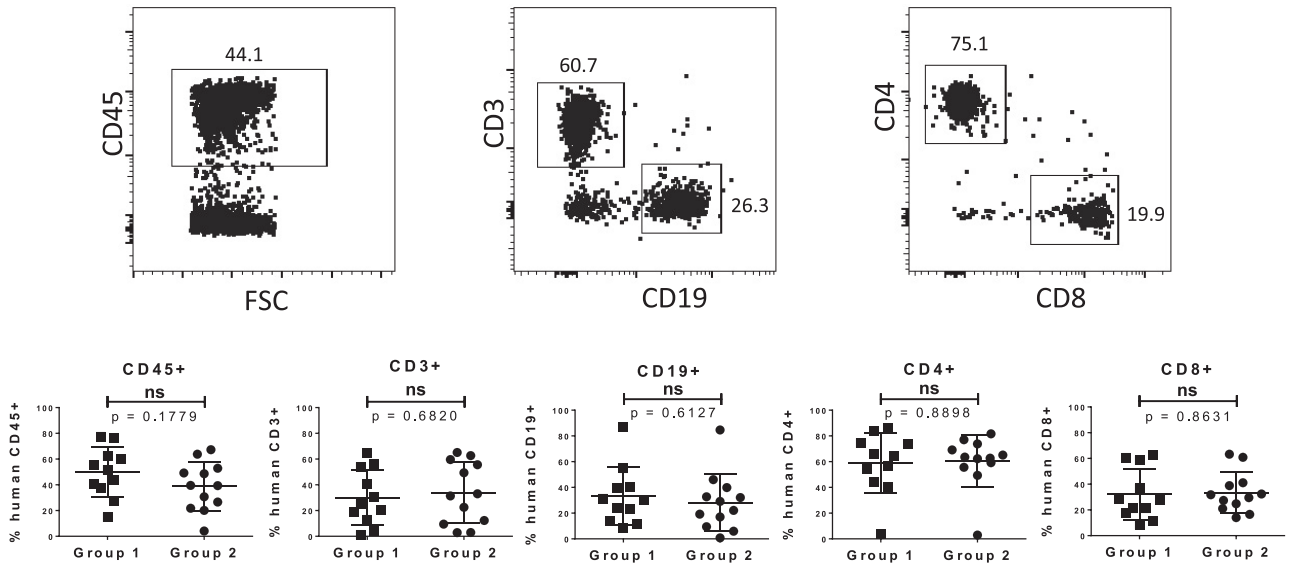

Figure 2. Human Hematopoietic Cell Reconstitution in Humanized Mice

Reconstitution of multi-lineage human hematopoietic cells was analyzed in peripheral blood in humanized mice 10 weeks after $CD34^+$ HSPC injection in neonatal NSG mice. Representative flow plots show the human $CD45^+$ hematopoietic population with forward scatter (FSC), human $CD3^+$ T lymphocyte and $CD19^+$ B lymphocyte population, and $CD4^+$ T lymphocyte and $CD8^+$ T lymphocyte population (upper panels). Humanized NSG mice were divided into 2 groups based on similar distributions of $\%CD45^+$, $\%CD3^+$, $\%CD19^+$, $\%CD4^+$, and $\%CD8^+$ human cell reconstitutions (lower panels). Group 1 mice were infected with CCR5-tropic HIV-1_{NFNSX} (200 ng of p24 Gag) 11 weeks after the $CD34^+$ HSPC transplant. Group 2 mice were mock. The horizontal bars represent the mean. The vertical bars represent the SE. NS, not significant.

no-shRNA control vector (mCherry marked). After vector transduction overnight, vector-transduced cells (0.5×10^6 cells) were mixed at a 1:1 ratio and transplanted intravenously via retro-orbital vein plexus, along with a cryopreserved, donor-matched human thymus tissue piece under the kidney capsule to create a human thymus implant, as previously described.[15,28,34,39,40] The efficiencies of vector transduction in $CD34^+$ HSPCs (n = 5) were determined by %EGFP expression at 72.92 ± 10.84 (% ± SD) and by %mCherry expression at 73.28 ± 18.98 (% ± SD) in an aliquot culture with cytokine stimulation (stem cell factor [SCF], interleukin-3 [IL-3], and IL-6 at 50 ng/mL each) 3 days after vector transduction.

Repopulation of Anti-HIV-1 Gene-Modified Human Hematopoietic Cells in HIV-1 Previously Infected Humanized Mice

Multi-lineage hematopoietic cell repopulation of $EGFP^+$ or $mCherry^+$ vector-modified cells was initially evaluated in peripheral blood 6 weeks after vector-modified $CD34^+$ HSPC transplant (Figure 3). $EGFP^+$ or $mCherry^+$ expressing cells were identified in human $CD45^+$ hematopoietic cells, $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes, and $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes in peripheral blood in both HIV-1-infected and non-HIV-1-infected humanized mice. There was statistically significant difference between %EGFP and %mCherry in human $CD4^+$ T lymphocytes in HIV-1 infected humanized mice ($p < 0.05$), but not in other lineages at this earliest time point ($p > 0.05$, paired Student's t test).

Selective Advantage of Anti-HIV-1 Gene Vector-Modified Human Hematopoietic Cells in HIV-1 Pre-infected Humanized Mice

Kinetics of %EGFP and %mCherry expressing $CD4^+$ T lymphocytes were monitored in peripheral blood from 6 to 10 weeks after vector-modified $CD34^+$ HSPC transplant. %EGFP increased more than %mCherry in $CD4^+$ T lymphocytes in the HIV-1 previously infected humanized mice ($p < 0.0001$, group-time interaction from a linear mixed-effects model) (Figure 4B). There were no significant differences in the kinetics of %EGFP and %mCherry expression in $CD4^+$ T lymphocytes in HIV-1-uninfected humanized mice (Figure 4A) and $CD8^+$ T lymphocytes in both uninfected and HIV-1 pre-infected humanized mice ($p > 0.05$, group-time interaction from a linear mixed-effects model) (Figures 4C and 4D). We then analyzed tissue repopulation 12 weeks after vector-modified $CD34^+$ HSPC transplant. The mean %EGFP was higher than the mean $\%mCherry^+$ in $CD4^+$ T lymphocytes in BM, lung, and gut in the HIV-1 previously infected humanized mice (Figure 5; Figure S1), suggesting that anti-HIV-1 dual sh1005/sh516 vector-modified $CD4^+$ T lymphocytes were positively selected by HIV-1-induced selective pressure in HIV-1 previously infected humanized mice. Viral load, however, was maintained in all HIV-1 previously infected humanized mice 12 weeks after anti-HIV-1 gene vector-modified $CD34^+$ HSPC transplant ($2.35 \times 10^7 \pm 4.10 \times 10^7$ copies/mL ± SD). Because each animal was reconstituted with not only $EGFP^+$ anti-HIV-1 gene vector-modified cells, but also cells marked with mCherry no-shRNA control vector and unmodified cells, the maintenance of viral load was expected. Altogether, these results demonstrated anti-HIV-1 dual sh1005/sh516 vector-modified HSPCs were able to engraft and

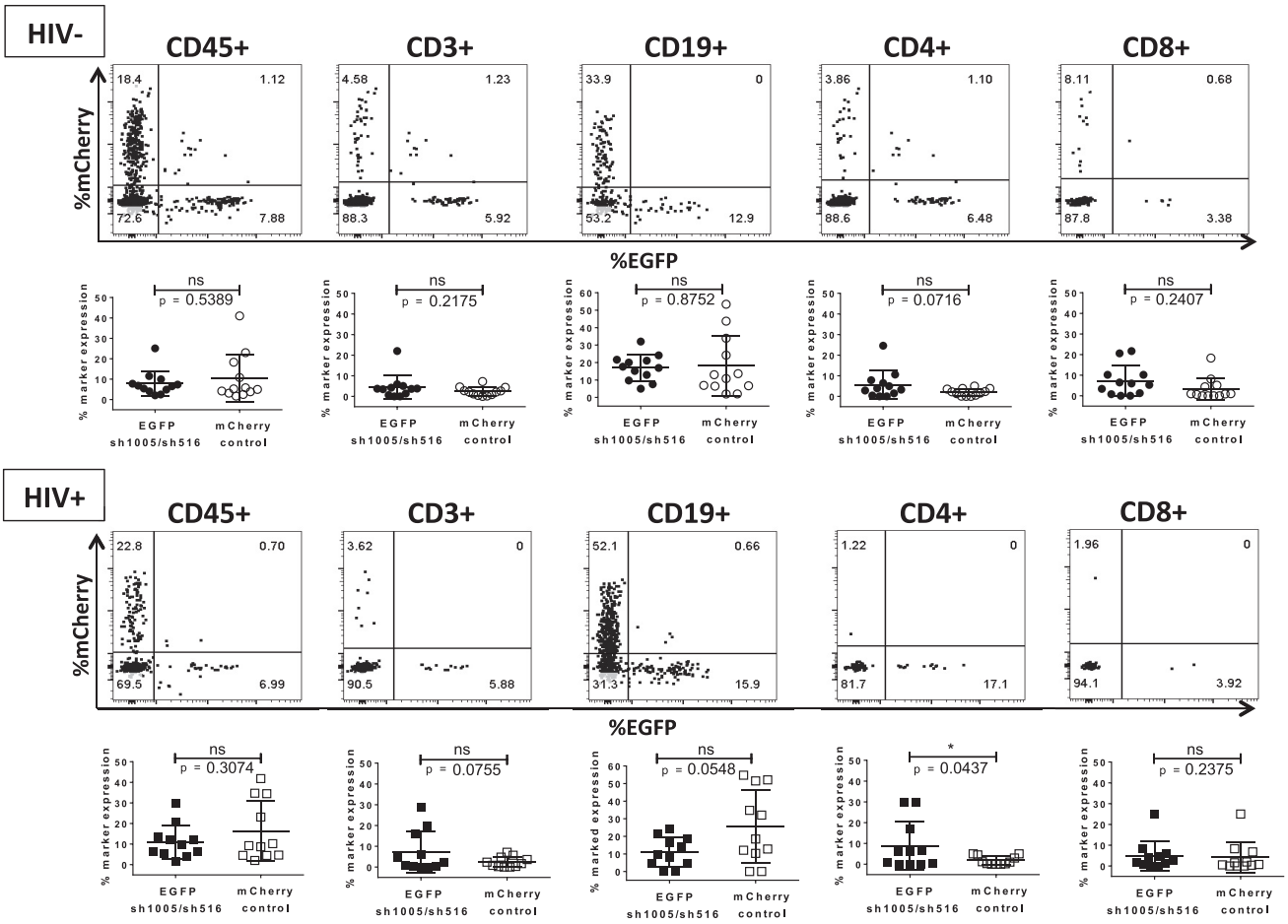

Figure 3. Repopulation of Vector-Modified Human Hematopoietic Cells in HIV-1 Previously Infected Humanized Mice

Humanized NSG mice (group 1 = HIV-1-infected) and (group 2 = HIV-1-uninfected) were preconditioned with busulfan (35 mg/kg) and transplanted with an equal mix of donor-matched anti-HIV-1 gene (sh1005/sh516) vector or no-shRNA control vector-modified fetal liver CD34$^+$ HSPCs 3 weeks after HIV-1 challenge with human thymus tissue transplant under the kidney capsule. Both %EGFP$^+$ and %mCherry$^+$ expression were analyzed in human CD45$^+$ hematopoietic cells, CD3$^+$ T lymphocytes, and CD19$^+$ B lymphocytes in peripheral blood 6 weeks after vector-modified CD34$^+$ HSPC transplant by flow cytometric analysis. A representative flow plot is shown on the top of each graph. The graph shows %EGFP$^+$ (closed) and %mCherry$^+$ (open) cells from all mice. The horizontal bars represent the mean. The vertical bars represent the SE. *p < 0.05. NS, not significant.

differentiate into mature lymphocytes in the presence of HIV-1 infection. The anti-HIV-1 dual sh1005/sh516 vector-modified CD4$^+$ T lymphocytes were positively selected over unprotected cells in the HIV-1 previously infected humanized mice.

CCR5 Downregulation

The level of CCR5 expression was compared in EGFP$^+$ and mCherry$^+$ CD4$^+$ T lymphocytes in tissues 12 weeks after vector-modified CD34$^+$ HSPC transplant. The level of CCR5 expression in the EGFP$^+$ CD4$^+$ T lymphocytes was reduced relative to that of the control mCherry$^+$ CD4$^+$ T lymphocytes in tissues in HIV-1-uninfected humanized mice (Figure 6; Figure S2), similar to our previously published results.[15,28] In humanized mice previously infected with HIV-1, CCR5 expression was reduced in EGFP$^+$ CD4$^+$ T lymphocytes. In addition, CCR5 expression was reduced in control mCherry$^+$ CD4$^+$ T lymphocytes. This could be due to HIV-1-mediated depletion of unprotected mCherry$^+$ CD4$^+$ T lymphocytes in HIV-1 previously infected humanized mice. Therefore, we did not observe a difference in relative CCR5 expression levels in EGFP$^+$ and mCherry$^+$ CD4$^+$ T lymphocytes.

DISCUSSION

In this study, we developed a new method to test an anti-HIV-1 HSPC-based gene therapy in HIV-1 previously infected humanized mice. The practical clinical application of anti-HIV-1 HSPC-based gene therapy is to treat HIV-1-infected patients.[5,41–44] Thus, it is important to examine anti-HIV-1 HSPC-based gene therapy strategies in animals already infected with HIV-1.

In our new method, we first infected humanized mice with HIV-1. After the establishment HIV-1 infection, determined by viremia, HIV-1-infected humanized mice were treated with busulfan for myeloablative conditioning and transplanted with anti-HIV-1

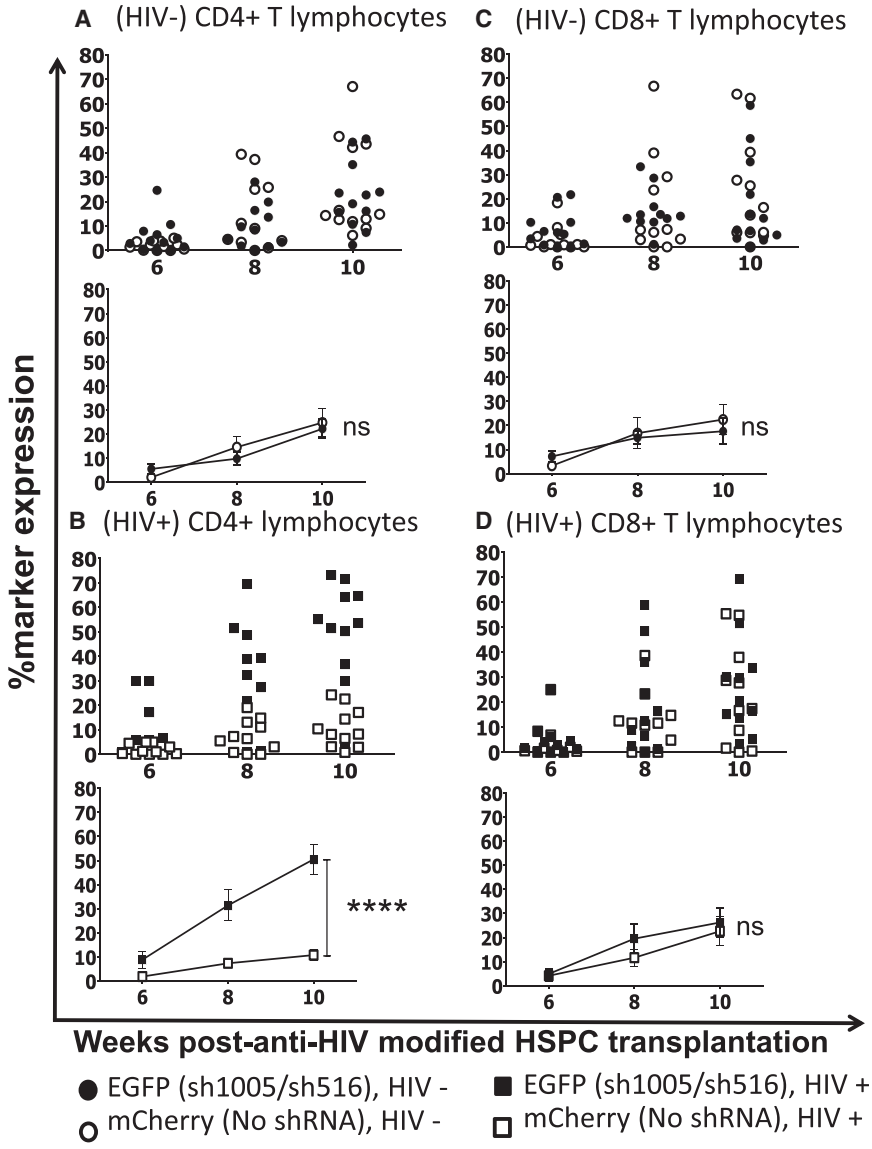

Figure 4. Selective Advantage of Anti-HIV-1 shRNA-Modified Human CD4⁺ T Lymphocytes in Peripheral Blood in HIV-1 Pre-infected Humanized Mice

Kinetics of %EGFP⁺ and %mCherry⁺ populations were compared in human peripheral blood CD4⁺ T lymphocytes in (A) HIV-1-uninfected and (B) HIV-1-infected humanized mice and in CD8⁺ T lymphocytes in (C) HIV-1-uninfected and (D) HIV-1-infected humanized mice from 6 to 10 weeks after vector-modified CD34⁺ HSPC transplant. The upper panels show data from all mice. The lower panels show line graphs for the mean percentages of EGFP⁺ and mCherry⁺ CD4⁺ T lymphocytes. The horizontal bars represent the mean. The vertical bars represent the SE. A linear mixed-effects model was used to evaluate statistical differences in log-transformed EGFP and mCherry marker levels over time across HIV-1 status. ****$p < 0.0001$. NS, not significant.

gene-modified HSPCs. Using this protocol, we were able to evaluate the engraftment of anti-HIV-1 gene vector-modified HSPCs, differentiation, tissue reconstitution, and selective advantage of anti-HIV-1 gene-modified cells in HIV-1 previously infected humanized mice. This new *in vivo* experimental method is more clinically relevant than previous experimental procedures in which anti-HIV-1 gene-modified HSPCs are first transplanted in uninfected humanized mice and subsequently challenged with HIV-1 to assess the efficacy of anti-HIV-1 reagents.

Human HSPCs were genetically engineered with the lentiviral vector expressing two anti-HIV-1 shRNAs: sh1005 directed to CCR5 and sh516 directed to HIV-1 LTR R region sequences.[15] The repopulation, CCR5 downregulation, and selective advantage of anti-HIV-1 gene-modified cells in humanized mice already infected with HIV-1 were comparable to our previously published post-infection results.[15,28,34] Our new results demonstrated that the anti-HIV-1 dual sh1005/sh516 vector-modified HSPCs successfully engrafted and differentiated into the HIV-1-resistant progeny CD4⁺ T cells and then selected from HIV-1-induced T cell loss in humanized mice already infected with HIV-1. To our knowledge, this is the first study to present successful engraftment of anti-HIV-1 gene-modified human HSPCs in HIV-1-infected humanized mice.

Accumulated evidence suggests that the current anti-retroviral drug therapy cannot provide a cure for AIDS.[45–47] Thus far, the first and only clinical cure of HIV/AIDS was achieved by transplants of CCR5Δ32/Δ32 homozygous HIV-1-resistant donor BM cells.[8–11] However, wider clinical application of such a protocol is impractical due to scarcity of HLA-matched allogeneic CCR5Δ32/Δ32 homozygous BM donors.[48,49] Autologous HSPC-based gene therapy can eliminate the need for finding HLA type-matched allogeneic CCR5Δ32/Δ32 homozygous BM donors. It has a potential to develop as a novel therapeutic strategy for an HIV-1 cure. To better evaluate the potential of anti-HIV-1 HSPC-based gene therapy strategies, it is desirable to examine in a clinically relevant setting. Our newly developed method to reconstitute HIV-1 already infected humanized mice with anti-HIV-1 gene-modified HSPCs will be a useful tool to investigate various anti-HIV-1 HSPC-based gene therapy strategies *in vivo* in an experimentally tractable, small animal model system.

MATERIALS AND METHODS

Human CD34⁺ HSPCs and Fetal Tissue

Human fetal thymuses and fetal livers were obtained from Advanced Bioscience Resources (ABR), FPA Women's Health, and the UCLA

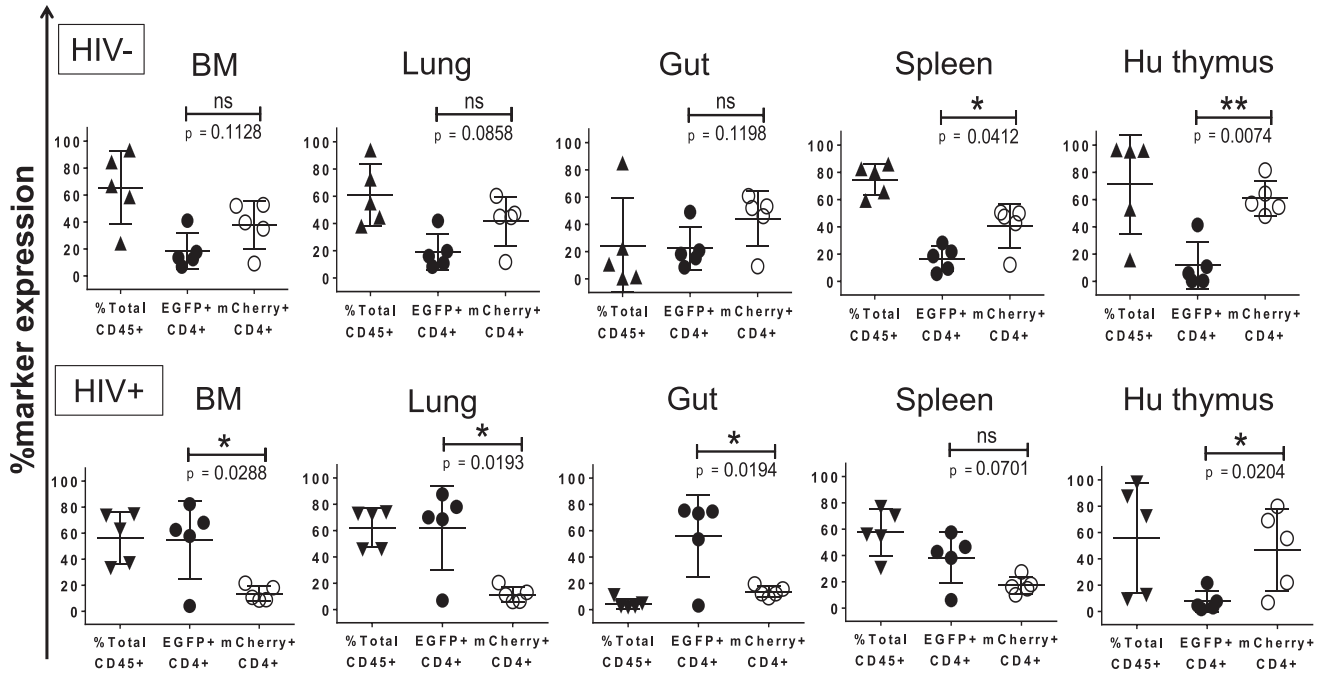

Figure 5. Selective Advantage of Anti-HIV-1 Gene shRNA-Modified Human CD4⁺ T Lymphocytes in Tissues in HIV-1 Previously Infected Humanized Mice
The levels of %EGFP⁺ and %mCherry⁺ in human CD4⁺ lymphocytes were compared in bone marrow (BM), lung, gut, spleen, and human thymus implant (Hu thymus) 12 weeks after vector-modified CD34⁺ HSPC transplant in mock (HIV-1-uninfected) and HIV-1 pre-infected (HIV-1-infected) mice. The horizontal bars represent the mean value. The vertical bars represent the SE. *$p < 0.05$, **$p < 0.01$, NS, not significant.

Center for AIDS Research (CFAR) Gene and Cellular Therapy Core. The UCLA institutional review board has determined that these tissues are not human subjects and do not require an institutional review board review, because fetal tissues were obtained without patient-identifying information from deceased fetuses. Written informed consent was obtained from patients for the use of tissues in research purposes. CD34⁺ HSPCs were isolated from fetal livers using anti-CD34⁺ magnetic bead-conjugated monoclonal antibodies (Miltenyi Biotec) and cryopreserved in Bambanker (Wako Chemical USA).[28] Human thymus pieces from the same donor were cryopreserved in 10% DMSO (Sigma-Aldrich) in human AB serum and stored in liquid nitrogen, as previously published.[39] They were thawed at 37°C in a water bath before use.

Humanized Mice

NSG mice were maintained at the UCLA CFAR Humanized Mouse Core laboratory in accordance with a protocol approved by the UCLA Animal Research Committee. All experiments conform to all relevant regulatory standards. Neonatal NSG mice (1–3 days old) were irradiated (125 cGy) and transplanted with human fetal liver CD34⁺ HSPCs (0.5×10^5–1.0×10^5 cells per mouse) by intrahepatic injection.

HIV-1 Infection

CCR5-tropic HIV-1_{NFNSX} stocks were prepared by a calcium phosphate plasmid DNA transfection method as previously described.[28] Humanized NSG mice were injected with HIV-1_{NFNSX} (200 ng of p24 Gag) via the retro-orbital vein plexus using a 27-gauge needle.

Viral Load Assay

Levels of HIV-1 RNA in plasma of infected humanized mice were determined by RT-PCR assay. 100 μL of whole blood was harvested via the retro-orbital vein plexus 3 weeks after HIV-1 injection. Approximately 50 μL of plasma was separated from peripheral blood and stored at −80°C until use. Viral RNA was isolated with a QIAamp viral RNA mini kit (QIAGEN). The RNA was eluted in 25 μL of RNase-free water, and 5 μL of elution was applied for qRT-PCR using an iScript One-step RT-PCR kit (Bio-Rad), with the following primers and probe specific to HIV-1_{NFNSX} gag region: primer sequence 1, 5′-CCCTACCAGCATTCTGGACATAAG-3′; primer sequence 2, 5′-GCTTGCTCGGCTCTTAGAGTT-3′; and probe 5′-FAM-ACAAGGACCAAAGGAACCCTT-BHQ1-3′. With these primers and probe, HIV-1 RNA can be quantitatively detected from 10^3 to 10^8 copies/mL.

Lentiviral Vector Production

Vesicular stomatitis virus G protein (VSVG)-pseudotyped lentiviral vector stocks were produced by calcium phosphate-mediated transient transfection of HEK293T cells, as previously described.[15,28,34] Vector stocks were titered on HEK293T cells

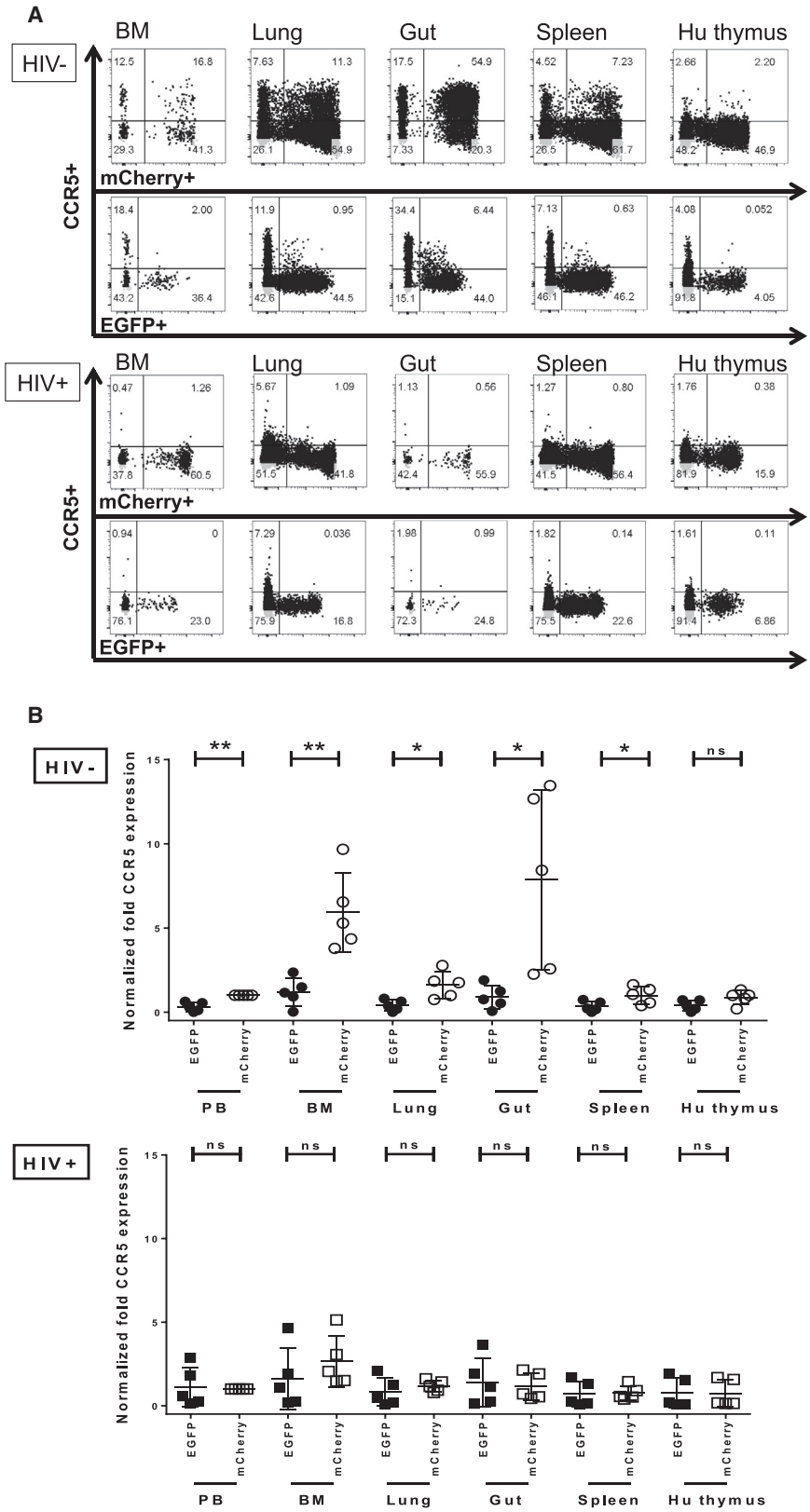

Figure 6. CCR5 Downregulation in Human CD4⁺ T Lymphocytes in Tissues

The level of CCR5 expression was compared in EGFP⁺ and mCherry⁺ human CD4⁺ T lymphocytes in multiple lymphoid tissues 12 weeks after vector-modified CD34⁺ HSPC transplant. (A) Representative data showing CCR5 downregulation in bone marrow (BM), lung, gut, spleen, and human thymus implant (Hu thymus) under the kidney capsule from the mock (HIV-1-uninfected) humanized mouse (upper panels) and the HIV-1 previously infected (HIV-1-infected) humanized mouse (lower panels). (B) CCR5 expression was compared in EGFP⁺ and mCherry⁺ CD4⁺ T lymphocytes from all mice. We normalized the CCR5 expression level using the mean CCR5 expression in mCherry⁺ cells from peripheral blood (PB) as 1, using the same method as in our previous publication.[28] The horizontal bars represent the mean. The vertical bars represent the SE. *p < 0.05. **p < 0.01. NS, not significant.

based on EGFP or mCherry expression analyzed by flow cytometric analysis.

Lentiviral Vector Transduction

The cryopreserved, fetal liver-derived $CD34^+$ HSPCs (0.5×10^6) were thawed and seeded into 20 μg/mL RetroNectin (Clontech Laboratories)-coated plates with 2% BSA (Sigma-Aldrich) in Yssel's medium (GEMINI Bio Products). After 1 hr of incubation, cells were transduced with either anti-HIV-1 dual sh1005/sh516 (MOI = 3) or no-shRNA control lentiviral vector (MOI = 1) overnight without cytokine stimulations to achieve the similar transduction efficiency measured by %EGFP or %mCherry expression, respectively. An aliquot of each transduced $CD34^+$ HSPC was cultured in RPMI 1640 (Gibco) with 10% fetal bovine serum (HyClone), supplemented with cytokine stimulations (SCF, IL-3, and IL-6; all three from PeproTech) at a concentration of 50 ng/mL for 3 days. The efficiencies of vector transduction were evaluated by flow cytometry (Fortessa flow cytometers, BD Biosciences). After transduction, vector-transduced $CD34^+$ HSPCs were mixed at a 1:1 ratio for transplantation into mice.

Transplantation of Anti-HIV-1 Gene-Modified $CD34^+$ HSPCs and Thymus Implantation in Humanized Mice Already Infected with HIV-1

HIV-1-infected and mock-infected humanized NSG mice were myeloablated by an injection of busulfan (35 mg/kg) (Sigma-Aldrich) in the peritoneal cavity one day before transplant. An equal mixture of vector-transduced $CD34^+$ HSPCs (0.5×10^6) was solidified with 5 μL of Matrigel (BD Biosciences). $CD34^-$ cells (4.5×10^6) were also mixed in the Matrigel as feeder cells. The Matrigel-solidified cell mix was implanted with a piece of thymus under the kidney capsule. On the same day, mice were injected with the vector-transduced human $CD34^+$ HSPCs (0.5×10^6) using a 27-gauge needle through the retro-orbital vein plexus.

Flow Cytometry

Isolation of peripheral blood mononuclear cells (PBMCs) and cells from the BM, lung, gut, spleen, and human thymus implant were described previously.[28] Peripheral blood- and tissue-derived mononuclear cells were stained with monoclonal antibodies to human CD45-eFluor 450 (HI30, eBioscience), CD3-APC H7 (SK7, Pharmingen), CD4-APC (OKT4, eBioscience), and CD8-PerCP Cy5.5 (SK1, BioLegend), CD19-Brilliant Violet 605 (HIB19, BD Horizon), and CCR5-PECy7 (2D7, Pharmingen). Red blood cells were lysed with red cell lysis buffer after cell surface marker staining. Stained cells were fixed with 1% formaldehyde in PBS and examined with Fortessa flow cytometers (BD Biosciences). The data were analyzed by FlowJo v.10 (Tree Star) software.

Statistical Analysis

A linear mixed-effects model was used to evaluate differences in log-transformed EGFP or mCherry marker intensity levels by HIV status at (1) baseline and (2) over time in Figure 4. The difference between groups and time was evaluated using a group-time interaction, and the p value for this interaction was calculated using a likelihood ratio test comparing the models with and without the interaction term. Given that there were three time points, time was modeled as a categorical variable. A compound symmetry correlation structure was used for all models. The paired Student's t test was used for other statistical analysis. Statistical significance was evaluated as $^*p < 0.05$. We indicate other significance levels as follows: $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Statistical analyses were performed using GraphPad Prism.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at https://doi.org/10.1016/j.omtm.2017.11.008.

AUTHOR CONTRIBUTIONS

D.S.A., S.S., and I.S.Y.C. designed experiments. W.K., S.S., R.C., J.W., P.K., Y.X., Y.J., J.L., S.K., H.A., A.P.P., and M.K. performed experiments and analyzed data. D.S.A., S.S., and W.K. wrote the paper.

CONFLICTS OF INTEREST

The authors declare no competing conflicts of interests.

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